

.alpha.I.SIGMA.I/.beta.I.SIGMA.I spectrin and Ab1 tyrosine kinase but not against .alpha.II/.beta.II spectrin colocalized with the overexpressed green fluorescent protein. Based on the conservation of the spectrin SH3-SH3-binding protein. Based on the conservation of the spectrin SH3 binding site within members of this protein family and published interactions, a general mechanism of interactions of tyrosine kinases with the spectrin-based membrane skeleton is proposed.

L6 ANSWER 4 OF 4 CA COPYRIGHT 2002 ACS
TI p53 is phosphorylated in vitro and in vivo by the delta and epsilon isoforms of casein kinase 1 and enhances the level of casein kinase 1 delta in response to topoisomerase-directed drugs
AU Knippschild, U.; Milne, D. M.; Campbell, L. E.; Demaggio, A. J.; Christenson, E.; Hoekstra, M. F.; Meek, D. W.
SO Oncogene (1997), 15(14), 1727-1736
CODEN: ONCNES; ISSN: 0950-9232
PY 1997
AB The p53 tumor suppressor protein plays a key role in the integration of stress signals. Multi-site phosphorylation of p53 may play an integral part in the transmission of these signals and is catalyzed by many different protein kinases including an unidentified p53-N-terminus-targeted protein kinase (p53NK) which phosphorylates a group of sites at the N-terminus of the protein. In this paper, the authors present evidence that the delta and epsilon isoforms of casein kinase 1 (CK1.delta. and CK1.epsilon.) show identical features to p53NK and can phosphorylate p53 both in vitro and in vivo. Recombinant, purified glutathione S-transferase (GST)-CK1.delta. and GST-CK1.epsilon. fusion proteins each phosphorylate p53 in vitro at serines 4, 6 and 9, the sites recognized by p53NK. Furthermore, p53NK (i) co-purifies with CK1.gamma./.epsilon., (ii) shares identical kinetic properties to CK1.delta./.epsilon., and (iii) is inhibited by a CK1.delta./.epsilon.-specific inhibitor (IC261). In addn., CK1.delta. is also present in purified preps. of p53NK as judged by immunoanal. using a CK1.delta.-specific monoclonal antibody. Treatment of murine SV3T3 cells with IC261 specifically blocked phosphorylation in vivo of the CK1.delta./.epsilon. phosphorylation sites in p53, indicating that p53 interacts physiol. with CK1.delta. and/or CK1.epsilon.. Similarly, over-expression of a green fluorescent protein (GFP)-CK1.delta. fusion protein led to hyper-phosphorylation of p53 at its N-terminus. Treatment of MethAp53ts cells with the topoisomerase-directed drugs etoposide or camptothecin led to increases in both CK1.delta.-mRNA and -protein levels in a manner dependent on the integrity of p53. These data suggest that p53 is phosphorylated by CK1.delta. and CK1.epsilon. and addnl. that there may be a regulatory feedback loop involving p53 and CK1.delta..

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FILE 'CA' ENTERED AT 14:32:42 ON 18 NOV 2002

L1 0 S PULL OUT ASSAY
L2 6129 S GREEN FLUORESCENT PROTEIN
L3 1986 S L2 AND (FUSION OR CONJUGATE)
L4 407 S L3 NOT 1999-2002/PY
L5 37 S L4 AND KINASE
L6 4 S L5 AND ISOFORMS

=> s l5 not 16
L7 33 L5 NOT L6

=> s 17 and cell?

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    835482 OUT
    263146 ASSAY
L1      0 PULL OUT ASSAY
        (PULL(W)OUT(W)ASSAY)

=> s green fluorescent protein
    195900 GREEN
    103345 FLUORESCENT
    1419936 PROTEIN
L2      6129 GREEN FLUORESCENT PROTEIN
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    203744 FUSION
    50025 CONJUGATE
L3      1986 L2 AND (FUSION OR CONJUGATE)

=> s l3 not 1999-2002/py
    2832753 1999-2002/PY
L4      407 L3 NOT 1999-2002/PY

=> s l4 and kinase
    177039 KINASE
L5      37 L4 AND KINASE

=> s l5 and isoforms
    34303 ISOFORMS
L6      4 L5 AND ISOFORMS

=> d 16 1-4 ti au so py ab

L6      ANSWER 1 OF 4 CA COPYRIGHT 2002 ACS
TI      Subspecies-specific targeting mechanism of protein kinase C
AU      Shirai, Yasuhito; Sakai, Norio; Saito, Naoaki
SO      Japanese Journal of Pharmacology (1998), 78(4), 411-417
CODEN: JJPAAZ; ISSN: 0021-5198
PY      1998
AB      A review with 30 refs. To clarify the subspecies-specific functions of protein kinase C (PKC), we constructed cDNAs encoding .gamma.-, .epsilon.- and .delta.-PKC fused with green fluorescent protein (GFP). All fusion proteins had enzymol. and immunol. characteristics similar to those of native PKCs. When expressed in CHO-K1 cells, each fusion protein showed a specific subcellular localization. Their translocations induced by various stimulation were also diverse. For example, ATP translocated .gamma.-, .epsilon.- and .delta.-PKC-GFP in the cytoplasm to the plasma membrane within 30 s with a return to the cytoplasm in 3 min, whereas TPA induced slow and irreversible translocation of all subspecies to the plasma membrane. Fatty acids also induced the translocation of .gamma.- and .epsilon.-PKC-GFP, but the two PKC subspecies showed distinct translocation and sensitivity to various fatty acids. Furthermore, we revealed that the PKC translocation requires neither the kinase activity of PKC nor its assocn. with cytoskeletal proteins such as F-actin. These results indicate that each subspecies has a spatially and temporally different targeting mechanism that depends on the extracellular and intracellular signals, contributing to the subspecies-specific functions of PKC. These remarkable findings also indicate that a system for monitoring the PKC translocation is a powerful tool for investigating the subspecies-specific functions of PKCs and mechanism of its translocation.
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WEST Search History

DATE: Monday, November 18, 2002

<u>Set Name</u>	<u>Query</u>	<u>Hit Count</u>	<u>Set Name</u>
side by side			result set
<i>DB=USPT,PGPB,JPAB,EPAB,DWPI,TDBD; PLUR=YES; OP=OR</i>			
L15	L14 and plasma membrane	340919	L15
L14	L13 and eukaryotic	238	L14
L13	L12 and gfp	239	L13
L12	L8 and kinase	1951	L12
L11	L10 and kinase	22660	L11
L10	L9 and plasma membrane	364423	L10
L9	L8 and green fluorescent protein	427373	L9
L8	L7 and heterologous adj4 (conjugate or fusion)	2226	L8
L7	L6 and inter\$6	137537	L7
L6	protein protein	283603	L6
L5	L4 and heterologous	6857	L5
L4	L3 and (conjugate or fusion)adj4 protein	9499	L4
L3	L2 and yeast	15543	L3
L2	L1 and kinase	30364	L2
L1	green floorescent protein	565538	L1

END OF SEARCH HISTORY

Christenson, E.; Hoekstra, M. F.; Meek, D. W.
SO Oncogene (1997), 15(14), 1727-1736
CODEN: ONCNES; ISSN: 0950-9232

PY 1997

AB The p53 tumor suppressor protein plays a key role in the integration of stress signals. Multi-site phosphorylation of p53 may play an integral part in the transmission of these signals and is catalyzed by many different protein kinases including an unidentified p53-N-terminus-targeted protein kinase (p53NK) which phosphorylates a group of sites at the N-terminus of the protein. In this paper, the authors present evidence that the delta and epsilon isoforms of casein kinase 1 (CK1.delta. and CK1.epsilon.) show identical features to p53NK and can phosphorylate p53 both in vitro and in vivo. Recombinant, purified glutathione S-transferase (GST)-CK1.delta. and GST-CK1.epsilon. fusion proteins each phosphorylate p53 in vitro at serines 4, 6 and 9, the sites recognized by p53NK. Furthermore, p53NK (i) co-purifies with CK1.gamma./.epsilon., (ii) shares identical kinetic properties to CK1.delta./.epsilon., and (iii) is inhibited by a CK1.delta./.epsilon.-specific inhibitor (IC261). In addn., CK1.delta. is also present in purified preps. of p53NK as judged by immunoanal. using a CK1.delta.-specific monoclonal antibody. Treatment of murine SV3T3 cells with IC261 specifically blocked phosphorylation in vivo of the CK1.delta./.epsilon. phosphorylation sites in p53, indicating that p53 interacts physiol. with CK1.delta. and/or CK1.epsilon.. Similarly, over-expression of a green fluorescent protein (GFP)-CK1.delta. fusion protein led to hyper-phosphorylation of p53 at its N-terminus. Treatment of MethAp53ts cells with the topoisomerase-directed drugs etoposide or camptothecin led to increases in both CK1.delta.-mRNA and -protein levels in a manner dependent on the integrity of p53. These data suggest that p53 is phosphorylated by CK1.delta. and CK1.epsilon. and addnl. that there may be a regulatory feedback loop involving p53 and CK1.delta..

AB A review with 30 refs. To clarify the subspecies-specific functions of protein kinase C (PKC), we constructed cDNAs encoding .gamma.-, .epsilon.- and .delta.-PKC fused with green fluorescent protein (GFP). All fusion proteins had enzymol. and immunol. characteristics similar to those of native PKCs. When expressed in CHO-K1 cells, each fusion protein showed a specific subcellular localization. Their translocations induced by various stimulation were also diverse. For example, ATP translocated .gamma.-, .epsilon.- and .delta.-PKC-GFP in the cytoplasm to the plasma membrane within 30 s with a return to the cytoplasm in 3 min, whereas TPA induced slow and irreversible translocation of all subspecies to the plasma membrane. Fatty acids also induced the translocation of .gamma.- and .epsilon.-PKC-GFP, but the two PKC subspecies showed distinct translocation and sensitivity to various fatty acids. Furthermore, we revealed that the PKC translocation requires neither the kinase activity of PKC nor its assocn. with cytoskeletal proteins such as F-actin. These results indicate that each subspecies has a spatially and temporally different targeting mechanism that depends on the extracellular and intracellular signals, contributing to the subspecies-specific functions of PKC. These remarkable findings also indicate that a system for monitoring the PKC translocation is a powerful tool for investigating the subspecies-specific functions of PKCs and mechanism of its translocation.

L6 ANSWER 2 OF 4 CA COPYRIGHT 2002 ACS
TI Sequence characteristics, subcellular localization, and substrate specificity of DYRK-related kinases, a novel family of dual specificity protein kinases
AU Becker, Walter; Weber, Yvonne; Wetzel, Kristiane; Eirmbter, Klaus; Tejedor, Francisco J.; Joost, Hans-Georg
SO Journal of Biological Chemistry (1998), 273(40), 25893-25902
CODEN: JBCHA3; ISSN: 0021-9258
PY 1998
AB DYRK1 is a dual specificity protein kinase presumably involved in brain development. The kinase belongs to a new family of protein kinases comprising at least seven mammalian isoforms (DYRK1A, DYRK1B, DYRK1C, DYRK2, DYRK3, DYRK4A, and DYRK4B), the yeast homolog Yak1p, and the Drosophila kinase minibrain (MNB). In rat tissues, DYRK1A is expressed ubiquitously, whereas transcripts for DYRK1B, DYRK2, DYRK3, and DYRK4 were detected predominantly in testes of adult but not prepuberal rats. By fluorescence microscopy and subcellular fractionation, a green fluorescent protein (GFP) fusion protein of DYRK1A was found to accumulate in the nucleus of transfected COS-7 and HEK293 cells, whereas GFP-DYRK2 was predominantly detected in the cytoplasm. DYRK1A exhibited a punctate pattern of GFP fluorescence inside the nucleus and was co-purified with the nuclear matrix. Anal. of GFP-DYRK1A deletion constructs showed that the nuclear localization of DYRK1A was mediated by its nuclear targeting signal (amino acids 105-139) but that its characteristics subnuclear distribution depended on addnl. N-terminal elements (amino acids 1-104). When expressed in Escherichia coli, DYRK1A, DYRK2, DYRK3, MNB, and Yak1p catalyzed their autophosphorylation on tyrosine residues. The kinases differed in their substrate specificity in that DYRK2 and DYRK3, but not DYRK1A and MNB, catalyzed phosphorylation of histone H2B. The heterogeneity of their subcellular localization and substrate specificity suggests that the kinases are involved in different cellular functions.

L6 ANSWER 3 OF 4 CA COPYRIGHT 2002 ACS
TI Identification of a candidate human spectrin Src homology 3 domain-binding protein suggests a general mechanism of association of tyrosine kinases with the spectrin-based membrane skeleton
AU Ziemnicka-Kotula, Dorota; Xu, Jiliu; Gu, Hong; Potempaska, Anna; Kim, Kwang Soo; Jenkins, Edmund C.; Trenkner, Ekhart; Kotula, Leszek
SO Journal of Biological Chemistry (1998), 273(22), 13681-13692
CODEN: JBCHA3; ISSN: 0021-9258
PY 1998
AB Spectrin is a widely expressed protein with specific isoforms found in erythroid and nonerythroid cells. Spectrin contains an Src homol. 3 (SH3) domain of unknown function. A cDNA encoding a candidate spectrin SH3 domain-binding protein was identified by interaction screening of a human brain expression library using the human erythroid spectrin (.alpha.I) SH3 domain as a bait. Five isoforms of the .alpha.I SH3 domain-binding protein mRNA were identified in human brain. Mapping of SH3 binding regions revealed the presence of two .alpha.I SH3 domain binding regions and one Abl-SH3 domain binding region. The gene encoding the candidate spectrin SH3 domain-binding protein has been located to human chromosome 10p11.2 .fwdarw. p12. The gene belongs to a recently identified family of tyrosine kinase-binding proteins, and one of its isoforms is identical to e3B1, an eps8-binding protein (Biesova, Z., Piccoli, C., and Wong, W. T. (1997)Oncogene 14, 233-241). Overexpression of the green fluorescent protein fusion of the SH3 domain-binding protein in NIH3T3 cells resulted in cytoplasmic punctate fluorescence characteristic of the reticulovesicular system. This fluorescence pattern was similar to that obtained with the anti-human erythroid spectrin .alpha.I.SIGMA.I/.beta.I.SIGMA.I antibody in untransfected NIH3T3 cells; in addn., the anti-.alpha.I.SIGMA.I/.beta.I.SIGMA.I antibody also stained Golgi app. Immunofluorescence obtained using antibodies against

2499705 CELL?
L8 31 L7 AND CELL?

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L8 ANSWER 1 OF 31 CA COPYRIGHT 2002 ACS
TI GFP tagging reveals human Polo-like kinase 1 at the kinetochore/centromere region of mitotic chromosomes
AU Arnaud, L.; Pines, J.; Nigg, E. A.
SO Chromosoma (1998), 107(6-7), 424-429
CODEN: CHROAU; ISSN: 0009-5915
PY 1998
AB Polo-like kinases (Plks) have been implicated in various aspects of M-phase progression in organisms ranging from yeast to man. In vertebrates, Plks participate in centrosome maturation and spindle assembly, as well as the activation of the Cdk1/cyclin B complex. Moreover, Plks are required for the destruction of mitotic cyclins, indicating that they play an important role in the regulation of the ubiquitin-dependent proteolytic degrdn. machinery that controls exit from M-phase. Here, we have fused Green Fluorescent Protein (GFP) to the N-terminus of human Plk1, and expressed this chimeric construct in human cells. We found that GFP-Plk1 assocs. with centrosomes, the equatorial spindle midzone and the postmitotic bridge of dividing cells, confirming and extending previous results obtained with conventional immunofluorescence microscopy. In addn., however, we obsd. fluorescence emanating from the midbody between dividing cells, and from discrete dots assocd. with mitotic chromosomes. This latter staining pattern being reminiscent of centromeres, we performed double-labeling expts. with antibodies against the centromeric marker CENP-B, and reexamd. the subcellular localization of endogenous Plk1 using different fixation procedures. Our data clearly show that both GFP-tagged Plk1 and endogenous Plk1 assoc. with the kinetochore/centromere region of human mitotic chromosomes. This novel localization of Plk1 suggests that substrates and/or regulators of Plks may be found among kinetochore-assocd. proteins with important functions in chromosome segregation and/or spindle checkpoint mechanisms.

L8 ANSWER 2 OF 31 CA COPYRIGHT 2002 ACS
TI Endocytosis of functional epidermal growth factor receptor-green fluorescent protein chimera
AU Carter, Royston E.; Sorkin, Alexander
SO Journal of Biological Chemistry (1998), 273(52), 35000-35007
CODEN: JBCHA3; ISSN: 0021-9258
PY 1998
AB A chimera of the epidermal growth factor receptor (EGFR) and green fluorescent protein (GFP) has been engineered by fusing GFP to the C-terminus of EGFR. Data are provided to demonstrate that the GFP moiety does not affect the expected functioning of EGFR. EGFR-GFP becomes phosphorylated at tyrosine residues in response to EGF and is capable of phosphorylating endogenous substrates and initiating signaling cascades. EGF-dependent assocn. of the chimeric receptor with the clathrin adaptor protein AP-2, involved in endocytosis, and with Shc adaptor protein, which binds in close proximity to the fusion point, is not affected by the GFP moiety. Receptor down-regulation and internalization occur at rates similar to those in cells expressing wild-type EGFR. Western blot anal. reveals that lysosomal degrdn. of EGFR-GFP proceeds from the extracellular domain and that GFP is not preferentially cleaved. Time-dependent co-localization of EGFR-GFP and Texas Red-conjugated EGF in living cells using digital deconvolution microscopy demonstrates the trafficking of ligand-receptor complexes through the early and multivesicular endosomes followed by segregation of the ligand and receptor at the late stages of endocytosis. Time-lapse optical anal. of the early stages of endocytosis reveals localization of EGFR-GFP in the tubular-vesicular endosomal compartments.

Rapid dynamics of membrane movement and **fusion** within these compartments were obsd. This approach and the fidelity of the biochem. properties of the EGFR-GFP demonstrate that real-time visualization of trafficking and protein interactions of tyrosine **kinase** receptors in the presence or absence of the ligand are feasible.

L8 ANSWER 3 OF 31 CA COPYRIGHT 2002 ACS
TI The Treacher Collins syndrome (TCOF1) gene product, treacle, is targeted to the nucleolus by signals in its C-terminus
AU Winokur, Sara T.; Shiang, Rita
SO Human Molecular Genetics (1998), 7(12), 1947-1952
CODEN: HMGEE5; ISSN: 0964-6906
PY 1998
AB The TCOF1 gene product, treacle, responsible for the craniofacial disorder Treacher Collins syndrome, has been predicted to be a member of a class of nucleolar phosphoproteins based on its primary amino acid sequence. Treacle is a low complexity protein with ten repeating units of acidic and basic residues, each of which contains a large no. of putative casein kinase 2 and protein kinase C phosphorylation sites. In addn., the C-terminus of treacle contains multiple putative nuclear localization signals. The overall structure of treacle, as well as sequence similarity to several nucleolar phosphoproteins, predicts that treacle is a member of this class of proteins. Using **green fluorescent protein fusion** constructs with the full-length and deleted domains of the murine homolog of treacle, the authors demonstrate that the **cellular** localization of treacle is nucleolar. This localization is mediated by the last 41 residues of the C-terminus (residues 1262-1302). At least two functional nuclear localization signals have been identified in the protein, one between residues 1176 and 1270 and the second within the last 32 residues of the protein (1271-1302). The nucleolar localization signal is disrupted by two constructs that split the C-terminal region between residues 1270 and 1271. This study provides the first direct anal. of treacle and demonstrates that the protein involved in TCOF1 is a nucleolar protein.

L8 ANSWER 4 OF 31 CA COPYRIGHT 2002 ACS
TI Determinants of 5-lipoxygenase nuclear localization using **green fluorescent protein/5-lipoxygenase fusion** proteins
AU Chen, Xin-Sheng; Zhang, Ying-Yi; Funk, Colin D.
SO Journal of Biological Chemistry (1998), 273(47), 31237-31244
CODEN: JBCHA3; ISSN: 0021-9258
PY 1998
AB 5-Lipoxygenase catalyzes the first two steps in the biosynthesis of leukotrienes, potent extracellular mediators of inflammation and allergic disorders. The unanticipated observation of 5-lipoxygenase in the nucleus of some **cell** types including bone marrow-derived mast **cells** (Chen, X. S., Naumann, T. A., Kurre, U., Jenkins, N. A., Copeland, N. G., and Funk, C. D. (1995) J. Biol. Chem. 270, 17993-17999) has raised speculation about intranuclear actions of leukotrienes or the enzyme itself. To explore the entry of 5-lipoxygenase into the nucleus we have transfected various **cell** types with expression vectors encoding native 5-lipoxygenase and **green fluorescent protein/5-lipoxygenase (GFP-SLO)** fusion proteins. 5-Lipoxygenase and **green fluorescent protein** /5-lipoxygenase co-localized with the nuclear DNA stain Hoechst 33258 in each **cell** type. The three main basic regions of 5-lipoxygenase were incapable of acting as "classical" nuclear localization signal sequences. Mutations that abolished enzyme activity/nonheme iron resulted in proteins that would no longer enter the nucleus. An N-terminal 5-lipoxygenase fragment of 80 residues was sufficient for directing nuclear localization of **green fluorescent protein** but not cytosolic pyruvate **kinase**. The combined data suggest that 5-lipoxygenase enters the nucleus not by a classical

nuclear localization signal but by a non-conventional signal located in the predicted .beta.-barrel domain that may be masked by structural alterations.

L8 ANSWER 5 OF 31 CA COPYRIGHT 2002 ACS
TI Random insertion of GFP into the cAMP-dependent protein kinase regulatory subunit from Dictyostelium discoideum
AU Biondi, Ricardo M.; Baehler, Pascal J.; Reymond, Christophe D.; Veron, Michel
SO Nucleic Acids Research (1998), 26(21), 4946-4952
CODEN: NARHAD; ISSN: 0305-1048
PY 1998
AB The green fluorescent protein (GFP) is currently being used for diverse cellular biol. approaches, mainly as a protein tag or to monitor gene expression. Recently it has been shown that GFP can also be used to monitor the activation of second messenger pathways by the use of fluorescence resonance energy transfer (FRET) between two different GFP mutants fused to a Ca²⁺ sensor. We show here that GFP fusions can also be used to obtain information on regions essential for protein function. As FRET requires the two GFPs to be very close, N- or C-terminal fusion proteins will not generally produce FRET between two interacting proteins. In order to increase the probability of FRET, we decided to study the effect of random insertion of two GFP mutants into a protein of interest. We describe here a methodol. for random insertion of GFP into the cAMP-dependent protein kinase regulatory subunit using a bacterial expression vector. The selection and anal. of 120 green fluorescent colonies revealed that the insertions were distributed throughout the R coding region. 14 R/GFP fusion proteins were partially purified and characterized for cAMP binding, fluorescence and ability to inhibit PKA catalytic activity. This study reveals that GFP insertion only moderately disturbed the overall folding of the protein or the proper folding of another domain of the protein, as tested by cAMP binding capacity. Furthermore, three R subunits out of 14, which harbor a GFP inserted in the cAMP binding site B, inhibit PKA catalytic subunit in a cAMP-dependent manner. Random insertion of GFP within the R subunit sets the path to develop two-component FRET with the C subunit.

L8 ANSWER 6 OF 31 CA COPYRIGHT 2002 ACS
TI Characterization of a nuclear deformed epidermal autoregulatory factor-1 (DEAF-1)-related (NUDR) transcriptional regulator protein
AU Huggenvik, Jodi I.; Michelson, Rhett J.; Collard, Michael W.; Ziembra, Amy J.; Gurley, Paul; Mowen, Kerri A.
SO Molecular Endocrinology (1998), 12(10), 1619-1639
CODEN: MOENEN; ISSN: 0888-8809
PY 1998
AB A monkey kidney cDNA that encodes a nuclear regulatory factor was identified by expression and affinity binding to a synthetic retinoic acid response element (RARE) and was used to isolate human placental and rat germ cell cDNAs by hybridization. The cDNAs encode a 59-kDa protein [nuclear DEAF-1-related (NUDR)] which shows sequence similarity to the Drosophila Deformed epidermal autoregulatory factor-1 (DEAF-1), a nonhomeodomain cofactor of embryonic Deformed gene expression. Similarities to other proteins indicate five functional domains in NUDR including an alanine-rich region prevalent in developmental transcription factors, a domain found in the promyelocytic leukemia-assocd. SP100 proteins, and a zinc finger homol. domain assocd. with the AML1/MTG8 oncogene. Although NUDR mRNA displayed a wide tissue distribution in rats, elevated levels of protein were only obsd. in testicular germ cells, developing fetus, and transformed cell lines. Nuclear localization of NUDR was demonstrated by immunocytochem. and by a green fluorescent protein-NUDR fusion protein. Site-directed mutagenesis of a nuclear localization signal resulted in cytoplasmic localization of the protein and eliminated

NUDR-dependent transcriptional activation. Retinoic acid receptor Recombinant NUDR protein showed affinity for the RARE in mobility shifts; however it was efficiently displaced by retinoic acid receptor (RAR)/retinoid X receptor (RXR) complexes. In transient transfections, NUDR produced up to 26-fold inductions of a human proenkephalin promoter-reporter plasmid, with minimal effects on the promoters for prodynorphin or thymidine kinase. Placement of a RARE on the proenkephalin promoter increased NUDR-dependent activation to 41-fold, but this RARE-dependent increase was not transferable to a thymidine kinase promoter. Recombinant NUDR protein showed minimal binding affinity for proenkephalin promoter sequences, but was able to select DNA sequences from a random oligonucleotide library that had similar core-binding motifs (TTCG) as those recognized by DEAF-1. This motif is also present between the half-sites of several endogenous RAREs. The derived consensus- binding motif recognized by NUDR (TTCGGGNNTTCCGG) was confirmed by mobility shift and DNase I (DNase I) protection assays; however, the consensus sequence was also unable to confer NUDR-dependent transcriptional activation to the thymidine kinase promoter. These data suggests that NUDR may activate transcription independently of promoter binding, perhaps through protein-protein interaction with basal transcription factors, or by activation of secondary factors. The sequence and functional similarities between NUDR and DEAF-1 suggest that NUDR may also act as a cofactor to regulate the transcription of genes during fetal development or differentiation of testicular cells.

L8 ANSWER 7 OF 31 CA COPYRIGHT 2002 ACS
TI Inhibition of cyclic AMP-dependent kinase by expression of a protein kinase inhibitor/enhanced green fluorescent fusion protein attenuates angiotensin II-induced type 1 AT1 receptor mRNA down-regulation in vascular smooth muscle cells
AU Wang, Xiaofei; Murphy, T. J.
SO Molecular Pharmacology (1998), 54(3), 514-524
CODEN: MOPMA3; ISSN: 0026-895X
PY 1998
AB Expression of the angiotensin II type 1 receptor (AT1-R) mRNA in vascular smooth muscle cells (VSMC) is down-regulated by a variety of agonists, including growth factors, agonists of G.alpha.q protein-coupled receptors, and activators of adenylyl cyclase. To det. whether cAMP-dependent protein kinases (PKA) participates in AT1-R mRNA down-regulation controlled by multiple classes of receptors, a PKA inhibitor peptide (PKI.alpha.) was developed and expressed in rat VSMC as a fusion with the enhanced green fluorescent protein (eGFP). PKA activity elicited both by forskolin and angiotensin II is suppressed in cells expressing this fusion protein (PKI.alpha.-eGFP), but platelet-derived growth factor-BB does not stimulate PKA activity in this prepn. PKI.alpha.-eGFP expression fully inhibits the forskolin-stimulated down-regulation of AT1-R mRNA levels and blocks 50% of the effect elicited by angiotensin II. This indicates that PKA plays a substantial role in angiotensin II-stimulated AT1-R mRNA down-regulation. However, inhibition of PKA has no effect on AT1-R mRNA down-regulation caused by platelet-derived growth factor-BB. These findings show how agonists such as angiotensin II that are not normally considered as activators of PKA can use PKA-dependent processes to modulate gene expression. These findings also provide definitive evidence that PKA-dependent pathways are involved in modulation of AT1-R mRNA levels in VSMC.

L8 ANSWER 8 OF 31 CA COPYRIGHT 2002 ACS
TI Activation of the Janus kinase/signal transducer and activator of transcription pathway by osmotic shock
AU Gatsios, Petros; Terstegen, Lara; Schliess, Freimut; Haussinger, Dieter; Kerr, Iam M.; Heinrich, Peter C.; Graeve, Lutz
SO Journal of Biological Chemistry (1998), 273(36), 22962-22968
CODEN: JBCHA3; ISSN: 0021-9258

PY 1998

AB Numerous cytokines, growth, and differentiation factors elicit their intracellular responses via Janus tyrosine kinases (Jaks) and transcription factors of the STAT (signal transducer and activator of transcription) family. Addnl., environmental stress (UV light, heat, aniso-osmolarity, and radicals) has recently been shown to activate intracellular signaling cascades such as the stress-activated protein kinases and nuclear factor-.kappa.B. In this study, the authors demonstrate that in different **cell** lines a particular stress, namely hyperosmolarity, results in tyrosine phosphorylation of the Janus kinases Jak1, Jak2, and Tyk2 and in the activation of STAT1 and/or STAT3. Both transcription factors are phosphorylated at a specific tyrosine residue and translocation to the nucleus was demonstrated by the use of a **STAT3/green fluorescent protein fusion** protein. A prominent role for Jak1 in the activation of STATs by hypertonicity was demonstrated by the use of Jak-deficient **cell** lines. Stress-activated STAT1 and STAT3 transactivate a reporter gene contg. the acute-phase response element of the rat .alpha.2-macroglobulin promoter. Expts. using a diffusible solute suggest that not the increase in intracellular osmolarity but the resultant **cell shrinkage** is the trigger for Jak/STAT activation.

L8 ANSWER 9 OF 31 CA COPYRIGHT 2002 ACS

TI Functional regions of the human cytomegalovirus protein pUL97 involved in nuclear localization and phosphorylation of ganciclovir and pUL97 itself

AU Michel, Detlef; Schaarschmidt, Peter; Wunderlich, Kirsten; Heuschmid, Maria; Simoncini, Lavinia; Muhlberger, Dita; Zimmermann, Albert; Pavic, Ivica; Mertens, Thomas

SO Journal of General Virology (1998), 79(9), 2105-2112
CODEN: JGVIAY; ISSN: 0022-1317

PY 1998

AB In order to identify functional regions of the human cytomegalovirus protein pUL97 (i) different 5' fragments of the UL97 open reading frame (ORF) were fused to the coding region of the **green fluorescent protein** and (ii) recombinant vaccinia viruses (rVV) were generated carrying two full-length and 11 mutated UL97 ORFs. The results indicated the presence of an N-terminal region within pUL97 which changed the intracellular distribution of the **fusion proteins**. Protein pUL97 was localized in the nucleus, but not in the nucleoli, and was detected in the nuclear matrix fraction. Expression of all pUL97 mutants could be confirmed by Western blot anal. Protein pUL97-assocd. ganciclovir (GCV) phosphorylation in rVV-infected **cells**, detd. quant. by HPLC anal., was abolished completely using individual UL97 deletion mutants. Phosphorylation of full-length and some of the mutated pUL97 was detected in **cells** infected with rVVs. The UL97 constructs carrying point mutations from GCV-resistant HCMV isolates at positions 460M, 520H, 594V, and the 4 aa deletion 590AACR593, also resulted in decreased but not abolished phosphorylation of GCV in the rVV system, whereas the phosphorylation of pUL97 itself was not influenced. The rVV system is a suitable method for quant. testing the functional relevance of pUL97 mutations.

L8 ANSWER 10 OF 31 CA COPYRIGHT 2002 ACS

TI Three distinct mechanisms for translocation and activation of the .delta. subspecies of protein **kinase C**

AU Ohmori, Shiho; Shirai, Yasuhito; Sakai, Norio; Fujii, Motoko; Konishi, Hiroaki; Kikkawa, Ushio; Saito, Naoki

SO Molecular and Cellular Biology (1998), 18(9), 5263-5271
CODEN: MCEBD4; ISSN: 0270-7306

PY 1998

AB The authors expressed .delta. subspecies of protein **kinase C** (PKC-.delta.) fused with **green fluorescent protein** (GFP) in CHO-K1 **cells** and obsd. the movement of this **fusion protein** in living **cells** after 3 different

stimulations. The PKC-.delta.-GFP fusion protein had enzymol. characteristics very similar to those of the native PKC-.delta. and was present throughout the cytoplasm in CHO-K1 cells. ATP at 1 mM concn. caused a transient translocation of PKC-.delta.-GFP to the plasma membrane .apprx.30 s after the stimulation and subsequent retranslocation to the cytoplasm within 3 min. The phorbol ester, 12-O-tetradecanoylphorbol 13-acetate (TPA; 1 .mu.M), induced a slower translocation of PKC-.delta.-GFP, and the translocation was unidirectional. Concomitantly, the kinase activity of PKC-.delta.-GFP was increased by these 2 stimulations, when the kinase activity of the immunopptd. PKC-.delta.-GFP was measured in vitro in the absence of PKC activators such as phosphatidylserine and diacylglycerol. H2O2 (5 mM) failed to translocate PKC-.delta.-GFP but increased its kinase activity by >3-fold. PKC-.delta.-GFP was strongly tyrosine-phosphorylated when treated with H2O2, but was tyrosine phosphorylated not at all by ATP stimulation and only slightly by TPA treatment. Both TPA and ATP induced the translocation of PKC-.delta.-GFP even after treatment with H2O2. Simultaneous treatment with TPA and H2O2 further activated PKC-.delta.-GFP >5-fold. TPA treatment of cells overexpressing PKC-.delta.-GFP led to an increase in the no. of cells in the G2/M phase and of dikaryons, whereas stimulation with H2O2 increased the no. of cells in the S phase and induced no significant change in cell morphol. These results indicate that at least 3 different mechanisms are involved in the translocation and activation of PKC-.delta..

L8 ANSWER 11 OF 31 CA COPYRIGHT 2002 ACS
TI A novel GFPneo vector designed for the isolation and analysis of enhancer elements in transfected mammalian cells
AU Primig, Michael; Chang, Ted Hung-Tse; Buckingham, Margaret
SO Gene (1998), 215(1), 181-189
CODEN: GENED6; ISSN: 0378-1119
PY 1998
AB The authors have designed a new approach to the direct cloning and rapid anal. of mammalian enhancer elements by fusing green fluorescent protein and neomycinphosphotransferase under the control of a thymidine kinase minimal promoter. DNA fragments contg. known or potential enhancer elements can be inserted into a polylinker upstream of GFPneo and re-isolated from stably transfected cell lines by a direct transgene-specific polymerase chain reaction (PCR), for further anal. C2C12 muscle cells were transfected with four vectors contg. the GFPneo fusion gene regulated by the cytomegalovirus promoter, the myoD distal core enhancer and myoblast- and myotube-specific enhancers from the desmin gene. GFPneo shows robust epifluorescence by microscopy and flow cytometry and retains sufficient neo activity to permit selection of G418-resistant clones. The fluorescence signal pattern of GFPneo expressed under the control of the desmin enhancers mirrors their transcriptional profile during myogenic differentiation. This finding demonstrates the value of GFPneo as a tool to analyze differentiation stage-specific regulatory DNA elements in stably transfected mammalian cell lines. We were able to re-isolate the myoD enhancer mediating GFPneo expression from a stably transfected C2C12 clone by a transgene-specific PCR reaction, demonstrating the feasibility of using this new vector system for the isolation of regulatory sequences.

L8 ANSWER 12 OF 31 CA COPYRIGHT 2002 ACS
TI Cooperation of a single lysine mutation and a C-terminal domain in the cytoplasmic sequestration of the p53 protein
AU Liang, Shun-Hsin; Hong, David; Clarke, Michael F.
SO Journal of Biological Chemistry (1998), 273(31), 19817-19821
CODEN: JBCHA3; ISSN: 0021-9258
PY 1998
AB Cytoplasmic sequestration of the p53 tumor suppresser protein has been

proposed as a mechanism involved in abolishing p53 function. However, the mechanisms regulating p53 subcellular localization remain unclear. In this report, we analyzed the possible existence of cis-acting sequences involved in intracellular trafficking of the p53 protein. To study p53 trafficking, the jellyfish **green fluorescent protein** (GFP) was fused to the wild-type or mutated p53 proteins for fast and sensitive anal. of protein localization in human MCF-7 breast cancer, RKO colon cancer, and SAOS-2 sarcoma cells. The wild-type p53/GFP fusion protein was localized in the cytoplasm, the nucleus, or both compartments in a subset of the cells. Mutagenesis anal. demonstrated that a single amino acid mutation of Lys-305 (mt p53) caused cytoplasmic sequestration of the p53 protein in the MCF-7 and RKO cells, whereas the fusion protein was distributed in both the cytoplasm and the nucleus of SAOS-2 cells. In SAOS-2 cells, the mutant p53 was a less efficient inducer of p21/CIP1/WAF1 expression. Cytoplasmic sequestration of the mt p53 was dependent upon the C-terminal region (residues 326-355) of the protein. These results indicated the involvement of cis-acting sequences in the regulation of p53 subcellular localization. Lys-305 is needed for nuclear import of p53 protein, and amino acid residues 326-355 can sequester mt p53 in the cytoplasm.

L8 ANSWER 13 OF 31 CA COPYRIGHT 2002 ACS
TI Bcl-2, Raf-1 and mitochondrial regulation of apoptosis
AU Wang, Hong-Gang; Reed, John C.
SO BioFactors (1998), 8(1,2), 13-16
CODEN: BIFAEU; ISSN: 0951-6433
PY 1998
AB A review, with 15 refs. Raf-1 kinase was shown to bind via its catalytic domain (Cat) to Bcl-2 in a BH4 domain-dependent manner. Using a green fluorescent protein (GFP)-Raf-1 (Cat) fusion protein, Bcl-2, but not Bcl-2(.DELTA.BH4), was found to target Raf-1 to mitochondria in cells. Targeting Raf-1 (Cat) to mitochondrial membranes by fusing with the transmembrane domain of an outer mitochondrial membrane protein protected cells from apoptosis and resulted in phosphorylation of BAD protein, whereas plasma-membrane targeted Raf-1 failed to phosphorylate BAD and did not protect against cell death. Moreover, a Bcl-2-binding protein, BAG-1, was shown to not only bind Raf-1 but also increase the activity of this kinase through a protein-protein interaction. The findings suggest that Bcl-2 targets Raf-1 to mitochondria, allowing this kinase to contribute to cellular survival by phosphorylating BAD or possibly other protein substrates in the vicinity of Bcl-1. X ?

L8 ANSWER 14 OF 31 CA COPYRIGHT 2002 ACS
TI Real-time optical monitoring of ligand-mediated internalization of .alpha.1b-adrenoceptor with green fluorescent protein
AU Awaji, Takeo; Hirasawa, Akira; Kataoka, Masakazu; Shinoura, Hitomi; Nakayama, Yasuhisa; Sugawara, Tatsuo; Izumi, Shun-Ichiro; Tsujimoto, Gozoh
SO Molecular Endocrinology (1998), 12(8), 1099-1111
CODEN: MOENEN; ISSN: 0888-8809
PY 1998
AB The study of G protein-coupled receptor signal transduction and behavior in living cells is tech. difficult because of a lack of useful biol. reagents. The authors show here that a fully functional .alpha.1b-adrenoceptor tagged with the green fluorescent protein (.alpha.1bAR/GFP) can be used to det. the mol. mechanism of internalization of .alpha.1bAR/GFP in living cells. In mouse .alpha.T3 cells, .alpha.1bAR/GFP demonstrates strong, diffuse fluorescence along the plasma membrane when obsd. by confocal laser scanning microscope. The fluorescent receptor binds agonist and antagonist and stimulates phosphatidylinositol/Ca²⁺ signaling in a similar

fashion to the wild receptor. In addn., .alpha.1bAR/GFP can be internalized within minutes when exposed to agonist, and the subcellular redistribution of this receptor can be detd. by measurement of endogenous fluorescence. The phospholipase C inhibitor U73, 122, the protein kinase C activator PMA, and inhibitor staurosporine, and the Ca²⁺-ATPase inhibitor thapsigargin were used to examine the mechanism of agonist-promoted .alpha.1bAR/GFP redistribution. Agonist-promoted internalization of .alpha.1bAR/GFP was closely linked to phospholipase C activation and was dependent on protein kinase C activation, but was independent of the increase in intracellular free Ca²⁺ concn. This study demonstrated that real-time optical monitoring of the subcellular localization of .alpha.1bAR (as well as other G protein-coupled receptors) in living cells is feasible, and that this may provide a valuable system for further study of the biochem. mechanism(s) of agonist-induced receptor endocytosis.

L8 ANSWER 15 OF 31 CA COPYRIGHT 2002 ACS
TI MPF localization is controlled by nuclear export
AU Hagting, Anja; Karlsson, Christina; Clute, Paul; Jackman, Mark; Pines, Jonathon
SO EMBO Journal (1998), 17(14), 4127-4138
CODEN: EMJODG; ISSN: 0261-4189
PY 1998
AB In eukaryotes, mitosis is initiated by M phase-promoting factor (MPF), composed of B-type cyclins and their partner protein kinase, CDK1. In animal cells, MPF is cytoplasmic in interphase and is translocated into the nucleus after mitosis has begun, after which it assocs. with the mitotic app. until the cyclins are degraded in anaphase. Here, the authors used a fusion protein between human cyclin B1 and green fluorescent protein (GFP) to study this dynamic behavior in real time, in living cells. The authors found that when they injected cyclin B1-GFP, or cyclin B1-GFP bound to CDK1 (i.e., MPF), into interphase nuclei it was rapidly exported into the cytoplasm. Cyclin B1 nuclear export was blocked by leptomycin B, an inhibitor of the recently identified export factor, exportin 1 (CRM1). The nuclear export of MPF was mediated by a nuclear export sequence in cyclin B1, and an export-defective cyclin B1 accumulated in interphase nuclei. Therefore, during interphase MPF constantly shuttles between the nucleus and the cytoplasm, but the bulk of MPF is retained in the cytoplasm by rapid nuclear export. The authors found that a cyclin mutant with a defective nuclear export signal does not enhance premature mitosis caused by interfering with the regulatory phosphorylation of CDK1, but is more sensitive to inhibition by Wee1 kinase.

L8 ANSWER 16 OF 31 CA COPYRIGHT 2002 ACS
TI Leptomycin B-sensitive nuclear export of MAPKAP kinase 2 is regulated by phosphorylation
AU Engel, Katrin; Kotlyarov, Alexey; Gaestel, Matthias
SO EMBO Journal (1998), 17(12), 3363-3371
CODEN: EMJODG; ISSN: 0261-4189
PY 1998
AB To study the intracellular localization of MAPKAP kinase 2 (MK2), which carries a putative bipartite nuclear localization signal (NLS), we constructed a green fluorescent protein-MAPKAP kinase 2 fusion protein (GFP-MK2). In transfected cells, this protein is located predominantly in the nucleus; unexpectedly, upon stress, it rapidly translocates to the cytoplasm. This translocation can be blocked by the p38 MAP kinase inhibitor SB203580, indicating its regulation by phosphorylation. Mol. mimicry of MK2 phosphorylation at T317 in GFP-MK2 led to a mutant which is located almost exclusively in the cytoplasm of the cell, whereas the mutant T317A shows no stress-induced redistribution. Since leptomycin B, which inhibits the interaction of exportin 1 with the Rev-type leucine-rich nuclear export signal (NES),

blocks stress-dependent translocation of GFP-MK2, it is supposed that phosphorylation-induced export of the protein causes the translocation. We have identified the region responsible for nuclear export in MK2 which is partially overlapping with and C-terminal to the autoinhibitory motif. This region contains a cluster of hydrophobic amino acids in the characteristic spacing of a leucine-rich Rev-type NES which is necessary to direct GFP-MK2 to the cytoplasm. However, unlike the Rev-type NES, this region alone is not sufficient for nuclear export. The data obtained indicate that MK2 contains a constitutively active NLS and a stress-regulated signal for nuclear export.

L8 ANSWER 17 OF 31 CA COPYRIGHT 2002 ACS
TI Tyrosine 1101 of Tie2 is the major site of association of p85 and is required for activation of phosphatidylinositol 3-kinase and Akt
AU Kontos, Christopher D.; Stauffer, Thomas P.; Yang, Wen-Pin; York, John D.; Huang, Liwen; Blanar, Michael A.; Meyer, Tobias; Peters, Kevin G.
SO Molecular and Cellular Biology (1998), 18(7), 4131-4140
CODEN: MCEBD4; ISSN: 0270-7306
PY 1998
AB Tie2 is an endothelium-specific receptor tyrosine kinase that is required for both normal embryonic vascular development and tumor angiogenesis and is thought to play a role in vascular maintenance. However, the signaling pathways responsible for the function of Tie2 remain unknown. In this report, the authors demonstrate that the p85 subunit of phosphatidylinositol 3-kinase (PI3-kinase) assocs. with Tie2 and that this assocn. confers functional lipid kinase activity. Mutation of tyrosine 1101 of Tie2 abrogated p85 assocn. both in vitro and in vivo in yeast. Tie2 was found to activate PI3-kinase in vivo as demonstrated by direct measurement of increases in cellular phosphatidylinositol 3-phosphate and phosphatidylinositol 3,4-bisphosphate, by plasma membrane translocation of a green fluorescent protein-Akt pleckstrin homol. domain fusion protein, and by downstream activation of the Akt kinase. Activation of PI3-kinase was abrogated in these assays by mutation of Y1101 to phenylalanine, consistent with a requirement for this residue for p85 assocn. with Tie2. These results suggest that activation of PI3-kinase and Akt may in part account for Tie2's role in both embryonic vascular development and pathol. angiogenesis, and they are consistent with a role for Tie2 in endothelial cell survival.

L8 ANSWER 18 OF 31 CA COPYRIGHT 2002 ACS
TI A GFP-PKC fusion protein to investigate the mechanism of PKC down-regulation
AU Smart, Nicola; Goode, Nigel T.
SO Biochemical Society Transactions (1998), 26(2), S126
CODEN: BCSTB5; ISSN: 0300-5127
PY 1998
AB The red shifted variant green fluorescent protein (RSGFP) coding sequence was cloned into the thiamine-repressible PREP41 for expressing in *S. pombe*. PREP41-RSGFP is expressed in a subset of cells but the level of expression gives a fluorescent intensity which is insufficient to perform expts. in this study for investigating the mechanism of PKC down-regulation.

L8 ANSWER 19 OF 31 CA COPYRIGHT 2002 ACS
TI Using fluorescent proteins as reporters in fusion proteins in the monitoring of cellular processes
IN Siegal, Micah S.; Isacoff, Ehud Y.
SO PCT Int. Appl., 53 pp.
CODEN: PIXXD2
PY 1998
1998
AB A method of using fluorescent proteins as reporters in the monitoring of

intracellular events, esp. signal transduction chains, in vivo is described. The **fusion** proteins exist in one of two states, i.e. different conformations. In one the fluorescent moiety does not fluoresce, in the other, it does fluoresce. Changes in fluorescence can therefore be used to monitor the development of a process. A chimeric gene for a **fusion** protein of the ShH4 potassium channel with the fluorescence domain of **green fluorescent**

protein incorporated into the sixth transmembrane domain was constructed. The ion channel was a mutant form that abolished ionic current, but retained gating currents. Capped mRNA from the gene was injected into Xenopus oocytes and the relationships between membrane current, membrane voltage and fluorescent were examd. The protein was stable in the oocyte over a period of weeks and did not show bleaching over several minute of illumination. Fluorescence changes followed voltage activation closely, but were slower than movements of the gating charge.

L8 ANSWER 20 OF 31 CA COPYRIGHT 2002 ACS
TI Enhanced cytotoxicity of nucleoside analogs by overexpression of mitochondrial deoxyguanosine kinase in cancer cell lines
AU Zhu, Chaoyong; Johansson, Magnus; Permert, Johan; Karlsson, Anna
SO Journal of Biological Chemistry (1998), 273(24), 14707-14711
CODEN: JBCHA3; ISSN: 0021-9258
PY 1998
AB The cytotoxic anti-cancer purine nucleoside analogs 2-chloro-2'-deoxyadenosine (CdA), 9-.beta.-D-arabinofuranosylguanine (araG), and 2',2'-difluorodeoxyguanosine (dFdG) are phosphorylated by human mitochondrial deoxyguanosine kinase (dGK) in vitro. We overexpressed dGK as a **fusion** protein to the **green fluorescent protein** in the human pancreatic cancer cell lines PanC-1 and MIA PaCa-2 to det. the importance of dGK-mediated nucleoside analog phosphorylation. The transfected cells showed mitochondrial fluorescence patterns, and the mitochondrial locations of endogenous and overexpressed dGK were verified by Western blot anal. of cell exts. with polyclonal anti-dGK antibodies. The increase of dGK activity in the overexpressing cells was .apprx.4-fold. These cell lines exhibited increased sensitivity to CdA, araG, and dFdG as compared with the untransfected parent cell lines. This is, to our knowledge, the first demonstration of a correlation between the activity of a mitochondrial deoxyribonucleoside kinase and the cytotoxicity of nucleoside analogs. Our data imply that the dGK activity is rate-limiting for the efficacy of nucleoside analogs in the cell lines investigated.

L8 ANSWER 21 OF 31 CA COPYRIGHT 2002 ACS
TI Visualization of dynamic trafficking of a protein kinase C .beta.II/green fluorescent protein conjugate reveals differences in G protein-coupled receptor activation and desensitization
AU Feng, Xiao; Zhang, Jie; Barak, Larry S.; Meyer, Tobias; Caron, Marc G.; Hannun, Yusuf A.
SO Journal of Biological Chemistry (1998), 273(17), 10755-10762
CODEN: JBCHA3; ISSN: 0021-9258
PY 1998
AB Protein kinase C (PKC) links various extracellular signals to intracellular responses and is activated by diverse intracellular factors including diacylglycerol, Ca²⁺, and arachidonic acid. In this study, using a fully functional **green fluorescent** protein-conjugated PKC.beta.II (GFP-PKC.beta.II), we demonstrate a novel approach to study the dynamic redistribution of PKC in live cells in response to G protein-coupled receptor activation. Agonist-induced PKC translocation was rapid, transient, and selectively

mediated by the activation of Gq.alpha.- but not Gs.alpha.- or Gi.alpha.-coupled receptors. Interestingly, although the stimuli were continuously present, only one brief peak of PKC membrane translocation was obsd., consistent with rapid desensitization of the signaling pathway. Moreover, when GFP-PKC.beta.II was used to examine cross-talk between two Gq.alpha.-coupled receptors, angiotensin II type 1A receptor (AT1AR) and endothelin A receptor (ETAR), activation of ETARs resulted in a subsequent loss of AT1AR responsiveness, whereas stimulation of AT1ARs did not cause desensitization of the ETAR signaling. The development of GFP-PKC.beta.II has allowed not only the real time visualization of the dynamic PKC trafficking in live **cells** in response to physiol. stimuli but has also provided a direct and sensitive means in the assessment of activation and desensitization of receptors implicated in the phospholipase C signaling pathway.

L8 ANSWER 22 OF 31 CA COPYRIGHT 2002 ACS
TI Herpes simplex virus thymidine kinase-green fluorescent protein fusion gene: new tool for gene transfer studies and gene therapy
AU Loimas, Sami; Wahlfors, Jarmo; Janne, Juhani
SO BioTechniques (1998), 24(4), 614-618
CODEN: BTNQDO; ISSN: 0736-6205
PY 1998
AB Green fluorescent protein (GFP) and herpes simplex virus type-1 thymidine kinase (TK) are commonly used markers in gene transfer studies. The latter gene has also proven to be an effective tool in cancer "suicide" gene therapy. To facilitate rapid and reliable selection of **cells** expressing TK, we constructed a plasmid expressing a TK-green fluorescent protein fusion gene (TK-GFP). In this **fusion** gene, the expression of each component is coupled to one another, permitting accurate detn. of the percentage of **cells** expressing TK by detecting the green fluorescence produced by GFP. Transfection of the **fusion** plasmid to mammalian **cells** revealed that the construct is fully functional, making the **cells** both fluorescent and sensitive to ganciclovir.

L8 ANSWER 23 OF 31 CA COPYRIGHT 2002 ACS
TI Multisite phosphorylation and the nuclear localization of phosphatase inhibitor 2-green fluorescent protein fusion protein during S phase of the cell growth cycle
AU Kakinoki, Yasutaka; Somers, Jeremy; Brautigan, David L.
SO Journal of Biological Chemistry (1997), 272(51), 32308-32314
CODEN: JBCHA3; ISSN: 0021-9258
PY 1997
AB Human phosphatase inhibitor 2 (Inh2) is a phosphoprotein that complexes with type 1 protein phosphatase, and its expression peaks during S phase and mitosis during the **cell** cycle. Localization of Inh2 was visualized in HS68 human fibroblasts by fusing Inh2 to green fluorescent protein (GFP). During G1 phase, Inh2-GFP was localized in the cytoplasm, and as **cells** progressed into S phase, Inh2-GFP accumulated in the nucleus. Known phosphorylation sites of Inh2 at Thr-72, Ser-86, and Ser-120/121 were each replaced with alanine. None of the mutated Inh2-GFP proteins accumulated in the nucleus during S phase, indicating that all of these phosphorylation sites were required. Mutation of two lysine residues in a putative nuclear localization sequence in Inh2 also prevented the Inh2-GFP fusion protein from accumulating in the nucleus during S phase. Recombinant Inh2 was phosphorylated by kinases in cytosols prep'd. from G1 and S phase **cells**. The amt. of Inh2 kinase attributed to casein kinase 2, based on inhibition by heparin, increased 2.6-fold from G1 to S phase. In addn., kinases in G1 vs. S phase cytosols produced distinct Inh2 phosphopeptides. The results indicate that changes in phosphorylation of Inh2 are involved in intracellular redistribution of

the protein during the cell cycle.

L8 ANSWER 24 OF 31 CA COPYRIGHT 2002 ACS
TI Direct visualization of the translocation of the .gamma.-subspecies of
protein kinase C in living cells using fusion
proteins with green fluorescent protein
AU Sakai, Norio; Sasaki, Keiko; Ikegaki, Natsu; Shirai, Yasuhito; Ono,
Yoshitaka; Saito, Naoaki
SO Journal of Cell Biology (1997), 139(6), 1465-1476
CODEN: JCLBA3; ISSN: 0021-9525
PY 1997
AB We expressed the .gamma.-subspecies of protein kinase C
(.gamma.-PKC) fused with green fluorescent
protein (GFP) in various cell lines and obsd. the
movement of this fusion protein in living cells under
a confocal laser scanning fluorescent microscope. .gamma.-PKC-GFP
fusion protein had enzymol. properties very similar to that of
native .gamma.-PKC. The fluorescence of .gamma.-PKC-GFP was obsd.
throughout the cytoplasm in transiently transfected COS-7 cells.
Stimulation by an active phorbol ester (12-O-tetradecanoylphorbol
13-acetate [TPA]) but not by an inactive phorbol ester (4.alpha.-phorbol
12, 13-didecanoate) induced a significant translocation of .gamma.-PKC-GFP
from cytoplasm to the plasma membrane. A23187, a Ca²⁺ ionophore, induced
a more rapid translocation of .gamma.-PKC-GFP than TPA. The
A23187-induced translocation was abolished by elimination of extracellular
and intracellular Ca²⁺. TPA-induced translocation of .gamma.-PKC-GFP was
unidirected, while Ca²⁺ ionophore-induced translocation was reversible;
i.e., .gamma.-PKC-GFP translocated to the membrane returned to the cytosol
and finally accumulated as patchy dots on the plasma membrane. To
investigate the significance of C1 and C2 domains of .gamma.-PKC in
translocation, we expressed mutant .gamma.-PKC-GFP fusion
protein in which the two cysteine rich regions in the C1 region were
disrupted (designated as BS 238) or the C2 region was deleted (BS 239).
BS 238 mutant was translocated by Ca²⁺ ionophore but not by TPA. In
contrast, BS 239 mutant was translocated by TPA but not by Ca²⁺ ionophore.
To examine the translocation of .gamma.-PKC-GFP under physiol. conditions,
we expressed it in NG-108 cells, N-methyl-D-aspartate (NMDA)
receptor-transfected COS-7 cells, or CHO cells
expressing metabotropic glutamate receptor 1 (CHO/mGluR1 cells).
In NG-108 cells, K⁺ depolarization induced rapid translocation
of .gamma.-PKC-GFP. In NMDA receptor-transfected COS-7 cells,
application of NMDA plus glycine also translocated .gamma.-PKC-GFP.
Furthermore, rapid translocation and sequential retranslocation of
.gamma.-PKC-GFP were obsd. in CHO/mGluR1 cells on stimulation
with the receptor. Neither cytochalasin D nor colchicine affected the
translocation of .gamma.-PKC-GFP, indicating that translocation of
.gamma.-PKC was independent of actin and microtubule. .gamma.-PKC-GFP
fusion protein is a useful tool for investigating the mol.
mechanism of .gamma.-PKC translocation and the role of .gamma.-PKC in the
central nervous system.

L8 ANSWER 25 OF 31 CA COPYRIGHT 2002 ACS
TI A .beta.-arrestin/green fluorescent protein
biosensor for detecting G protein-coupled receptor activation
AU Barak, Larry S.; Ferguson, Stephen S. G.; Zhang, Jie; Caron, Marc G.
SO Journal of Biological Chemistry (1997), 272(44), 27497-27500
CODEN: JBCHA3; ISSN: 0021-9258
PY 1997
AB G protein-coupled receptors (GPCR) represent the single most important
drug targets for medical therapy, and information from genome sequencing
and genomic data bases has substantially accelerated their discovery. The
lack of a systematic approach either to identify the function of a new
GPCR or to assoc. it with a cognate ligand has added to the growing no. of
orphan receptors. In this work we provide a novel approach to this

problem using a .beta.-arrestin2/green fluorescent protein conjugate (.beta.arr2-GFP). It provides a real-time and single cell based assay to monitor GPCR activation and GPCR-G protein-coupled receptor kinase or GPCR-arrestin interactions. Confocal microscopy demonstrates the translocation of .beta.arr2-GFP to more than 15 different ligand-activated GPCRs. These data clearly support the common hypothesis that the .beta.-arrestin binding of an activated receptor is a convergent step of GPCR signaling, increase by 5-fold the no. of GPCRs known to interact with .beta.-arrestins, demonstrate that the cytosol is the predominant reservoir of biol. active .beta.-arrestins, and provide the first direct demonstration of the crit. importance of G protein-coupled receptor kinase phosphorylation to the biol. regulation of .beta.-arrestin activity and GPCR signal transduction in living cells. The use of .beta.arr2-GFP as a biosensor to recognize the activation of pharmacol. distinct GPCRs should accelerate the identification of orphan receptors and permit the optical study of their signal transduction biol. intractable to ordinary biochem. methods.

L8 ANSWER 26 OF 31 CA COPYRIGHT 2002 ACS
TI Human deoxycytidine kinase is located in the cell nucleus
AU Johansson, Magnus; Brismar, Sophia; Karlsson, Anna
SO Proceedings of the National Academy of Sciences of the United States of America (1997), 94(22), 11941-11945
CODEN: PNASA6; ISSN: 0027-8424
PY 1997
AB Human deoxyribonucleoside kinases are required for the pharmacol. activity of several clin. important anticancer and antiviral nucleoside analogs. Human deoxycytidine kinase and thymidine kinase 1 are described as cytosolic enzymes in the literature, whereas human deoxyguanosine kinase and thymidine kinase 2 are believed to be located in the mitochondria. We expressed the four human deoxyribonucleoside kinases as fusion proteins with the green fluorescent protein to study their intracellular locations in vivo. Our data showed that the human deoxycytidine kinase is located in the cell nucleus, and the human deoxyguanosine kinase is located in the mitochondria. The fusion proteins between green fluorescent protein and thymidine kinases 1 and 2 were both predominantly located in the cytosol. Site-directed mutagenesis of a putative nuclear targeting signal, identified in the primary structure of deoxycytidine kinase, completely abolished nuclear import of the protein. Reconstitution of a deoxycytidine kinase-deficient cell line with the wild-type nuclear or the mutant cytosolic enzymes both restored sensitivity toward anticancer nucleoside analogs. This paper reports that a deoxyribonucleoside kinase is located in the cell nucleus, and we discuss the implications for deoxyribonucleotide synthesis and phosphorylation of nucleoside analogs.

L8 ANSWER 27 OF 31 CA COPYRIGHT 2002 ACS
TI Novel fluorescent indicator proteins for monitoring free intracellular Ca²⁺
AU Persechini, Anthony; Lynch, Jennifer A.; Romoser, Valerie A.
SO Cell Calcium (1997), 22(3), 209-216
CODEN: CECADV; ISSN: 0143-4160
PY 1997
AB We have recently described a fluorescent indicator protein in which red- and blue-shifted variants of green fluorescent protein are joined by the calmodulin-binding sequence from smooth muscle myosin light chain kinase [Romoser V.A., Hinkle P.M., Persechini A. Detection in living cells of Ca²⁺-dependent changes in the fluorescence of an indicator composed of two green fluorescent protein variants linked by a

calmodulin-binding sequence. A new class of fluorescent indicators. J Biol Chem 1997; 272: 13270-13274]. The fluorescence emission of this protein at 505 nm (380 nm excitation) is reduced by .apprx.65% when (Ca²⁺)₄-calmodulin is bound, with a proportional increase in fluorescence emission at 440 nm. We have found that **fusion** of an engineered calmodulin, in which the C- and N-terminal EF hand pairs have been exchanged, to the C-terminus of this protein results in a novel indicator that responds directly to changes in the Ca²⁺ ion concn., with an apparent K_d value of 100 nM for Ca²⁺ in the presence of 0.5 mM Mg²⁺. The affinity of the indicator for Ca²⁺ can be decreased by altering the amino acid sequence of the calmodulin-binding sequence to weaken its interaction with the intrinsic calmodulin domain. The fluorescence emission of this indicator can be used to monitor physiol. changes in the free Ca²⁺ ion concn. in living **cells**.

L8 ANSWER 28 OF 31 CA COPYRIGHT 2002 ACS
TI AUT3, a serine/threonine **kinase** gene, is essential for autophagocytosis in *Saccharomyces cerevisiae*
AU Straub, Michael; Bredschneider, Monika; Thumm, Michael
SO Journal of Bacteriology (1997), 179(12), 3875-3883
CODEN: JOBAAY; ISSN: 0021-9193
PY 1997
AB Autophagocytosis is a starvation-induced process, carrying proteins destined for degrdn. to the lysosome. In the yeast *S. cerevisiae*, the autophagic process is visualized by the appearance of autophagic vesicles in the vacuoles of proteinase *yscB*-deficient strains during starvation. Aut3-1 mutant **cells** which exhibit a block in the autophagic process have been isolated previously. By using the drastically reduced sporulation frequency of homozygous aut3-1 diploid **cells**, the AUT3 gene was cloned by complementation. The Aut3 protein consists of 897 amino acids. The amino-terminal part of the protein shows significant homologies to serine/threonine kinases. Aut3 null mutant **cells** are fully viable on rich media but show a reduced survival rate upon starvation. They are unable to accumulate autophagic vesicles in the vacuole during starvation. Starvation-induced vacuolar protein breakdown is almost completely impaired in aut3-deficient **cells**. Vacuolar morphol. and acidification are not influenced in aut3-deficient **cells**. Secretion of invertase, endocytic uptake of Lucifer yellow, and vacuolar protein sorting appear wild type like in aut3-deficient **cells**, suggesting autophagocytosis as a novel route for the transport of proteins from the cytosol to the vacuole. By using a **fusion** of Aut3p with green-fluorescent protein, Aut3p was localized to the cytosol.

L8 ANSWER 29 OF 31 CA COPYRIGHT 2002 ACS
TI Bcl-2 targets the protein **kinase** Raf-1 to mitochondria
AU Wang, Hong-Gang; Rapp, Ulf R.; Reed, John C.
SO Cell (Cambridge, Massachusetts) (1996), 87(4), 629-638
CODEN: CELLS5; ISSN: 0092-8674
PY 1996
AB A green fluorescent protein (GFP)-Raf-1 fusion protein was used to show that Bcl-2 can target this kinase to mitochondria. Active Raf-1 fused with targeting sequences from an outer mitochondrial membrane protein protected **cells** from apoptosis and resulted in phosphorylation of BAD, a proapoptotic Bcl-2 homolog. Plasma membrane-targeted Raf-1 did not protect from apoptosis and resulted in phosphorylation of ERK-1 and ERK-2. Untargeted active Raf-1 improved Bcl-2-mediated resistance to apoptosis, whereas a kinase-inactive Raf-1 mutant abrogated apoptosis suppression by Bcl-2. Bcl-2 can therefore target Raf-1 to mitochondrial membranes, allowing this kinase to phosphorylate BAD or possibly other protein substrates involved in apoptosis regulation.

L8 ANSWER 30 OF 31 CA COPYRIGHT 2002 ACS

TI Genetic interactions indicate a role for Mdglp and the SH3 domain protein Bem1p in linking the G-protein mediated yeast pheromone signalling pathway to regulators of **cell** polarity
AU Leberer, Ekkehard; Chenevert, Janet; Leeuw, Thomas; Harcus, Doreen; Herskowitz, Ira; Thomas, David Y.
SO Molecular & General Genetics (1996), 252(5), 608-621
CODEN: MGGEAE; ISSN: 0026-8925
PY 1996
AB The pheromone signal in the yeast *Saccharomyces cerevisiae* is transmitted by the .beta. and .gamma. subunits of the mating response G-protein. The STE20 gene, encoding a protein **kinase** required for pheromone signal transduction, has recently been identified in a genetic screen for high-gene-dosage suppressors of a partly defective G.beta. mutation. The same genetic screen identified BEM1, which encodes an SH3 domain protein required for polarized morphogenesis in response to pheromone, and a novel gene, designated MDG1 (multicopy suppressor of defective G-protein). The MDG1 gene was independently isolated in a search for multicopy suppressors of a bem1 mutation. The MDG1 gene encodes a predicted hydrophilic protein of 364 amino acids with a mol. wt. of 41 kDa that has no homol. with known proteins. A **fusion** of Mdglp with the **green fluorescent protein** from *Aequorea victoria* localizes to the plasma membrane, suggesting that Mdglp is an extrinsically bound membrane protein. Deletion of MDG1 causes sterility in **cells** in which the wild-type G.beta. has been replaced by partly defective G.beta. derivs. but does not cause any other obvious phenotypes. The mating defect of **cells** deleted for STE20 is partially suppressed by multiple copies of BEM1 and CDC42, which encodes a small GTP-binding protein that binds to Ste20p and is necessary for the development of **cell** polarity. Elevated levels of STE20 and BEM1 are capable of suppressing a temp.-sensitive mutation in CDC42. This complex network of genetic interactions points to a role for BEM1p and Mdglp in G-protein mediated signal transduction and indicates a functional linkage between components of the pheromone signalling pathway and regulators of **cell** polarity during yeast mating.

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TI The fission yeast sts5+ gene is required for maintenance of growth polarity and functionally interacts with protein **kinase** C and an osmosensing MAP **kinase** pathway
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AB **Cell** morphogenesis is a fundamental phenomenon that involves understanding a no. of biol. processes including the developmental program, polarity, and **cell** division. Fission yeast sts5 mutant **cells** are round rather than cylindrical, with cortical actin randomly dispersed. Genetic analyses demonstrate that the sts5+ gene is required for maintenance of **cell** shape during interphase when the **cell** normally exhibits polarized growth. The sts5 mutant is not defective in **cell** wall integrity. Deletion of ppe1+, which encodes a type 2A-like protein phosphatase, shows similar phenotypes to the sts5 mutant, and these 2 mutations are synthetically lethal. Multicopy plasmids contg. either the protein **kinase** C-like gene pck1+ or the protein tyrosine phosphatase pyp1+, an inhibitor of an osmosensing Sty1/Spc1 MAP-**kinase**, suppress the sts5 mutation. Consistent with this, the wis1 mutation, which is defective in a MAP-**kinase** kinase of the pathway, suppresses the sts5 mutation. The predicted sts5+ gene product exhibits sequence similarity to 2 yeast proteins, Dis3 and Ssd1, and a nematode protein, F46E8.6, where the former 2 yeast proteins have been shown to be involved in **cell** cycle control and **cell** morphogenesis. The sts5+ gene is not essential for **cell** viability, but is absolutely required for

polarized growth as the gene distribution showed the same phenotypes as those of the original mutants. Overexpression of the *sts5*⁺ gene resulted in altered **cell morphol.** and cortical actin in these overproducing **cells** was also abnormal, fainter, and often dispersed. Anti-*Sts5* antibody specifically detected a 130-kDa protein by western blotting. A **green fluorescent protein**-*Sts5* **fusion** protein localized in the cytoplasm with a discrete punctate pattern, suggesting that the *Sts5* protein is a component of a novel structure. These results have indicated that the *Sts5* protein is a crucial determinant of polarized growth and that it functionally interacts with the serine/threonine phosphatase, protein **kinase C**, and an osmosensing **MAP-kinase** to maintain **cell morphol.**